sisted nonetheless. The authors sequenced the virus DNA and proteins after eight weeks, and found that none of the initial SIV strain remained. The viruses had evolved from that first strain, and were able to escape being recognized by the CTLs. Changes occurred in the Tat peptide in all ten of the animals studied in detail. The rest of the virus appeared to be largely untouched by immune pressure. The authors' finding that the steady-state viral load was lower in animals with early, Tat-specific CTL responses attests to the important antiviral effect of these cells.

One of the most intriguing questions raised by these results is why the virus escaped by mutation in the Tat peptide, rather than the Gag peptide, given that the timing and magnitude of the CTL responses to these two peptides seemed to be similar. Perhaps Tat-specific CTLs are more effective at controlling the virus, and so impose a greater selection pressure on the virus. Alternatively, sequence changes may be better tolerated in certain regions of the viral proteins but constrained in others. For example, the Tat peptide is derived from a region of the Tat protein with no defined function, so it might not be damaging to the protein if this peptide were allowed to mutate.

These results have implications in the quest for an HIV vaccine. First, we can no longer look at CTL responses to a single viral peptide as a substitute for overall CTL function — instead, we need to consider the breadth of the immune response. Second, the results suggest that all CTL responses are not created equal, so we need to determine whether CTL specific for Tat and the other regulatory proteins are the most effective at controlling HIV infection. Finally, particularly in studies of acute infection, we need to look at the CTL responses to the infecting strain of virus, as opposed to a consensus, published sequence. The greater effort required to sequence the infecting strain will no doubt be rewarded by a much better understanding of where immune pressure is being applied.

So it seems that Tat-specific CTLs are a key part of the immune system's early response to SIV, raising the possibility that they might be important in the development of an HIV vaccine. Other results indicate that using Tat may be a promising way to stimulate the immune system[10]. A pre-existing Tat-specific immune response at the time of virus infection would be expected to inhibit virus more effectively than normal, and to prevent the high level of HIV replication that promotes mutations and immune escape (Fig. 1). This idea can now be tested by experimentally inducing the production of Tat-specific CTLs in macaques, and then introducing SIV. The value of animal models of disease is perhaps nowhere more clearly seen than in these studies[2]. ■

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Developmental biology

Post-expressionist flies

Sarah Bray and David Stein

The study of developmental biology is concerned with two main issues: the generation of cell diversity, and the organization of these diverse cells into beautifully patterned structures. These processes make use of relatively few, evolutionarily conserved cell-to-cell communication pathways. The mechanisms that coordinate the activities of these signalling pathways must be instrumental in fine-tuning the pattern of the developing organism. Over the past decade or so, emphasis has been placed on mechanisms of gene expression and its regulation as ways of coordinating cellular pathways. So it was exciting, at a recent meeting focusing on the fruitfly *Drosophila melanogaster**, to realize the variety of mechanisms that regulate the activity and subcellular localization of proteins in several signalling pathways.

Protein turnover is often neglected as a way of controlling cellular pathways, because most experimental methods detect steady-state levels of proteins in cells. But protein degradation appears to be important in the so-called Wingless and Hedgehog cell-to-cell signalling pathways. These pathways have several roles in the patterning of structures during *Drosophila* development. For example, they are involved in arranging the cuticle structures secreted by the embryonic epidermis (the outermost cell layer) and in shaping appendages. One long-standing mystery about Wingless activity in the epidermis is the asymmetric distribution of Wingless protein in each epidermal segment. Previous studies indicated that a barrier prevents the spread of Wingless to posterior parts of each segment[1]. But now it seems that the asymmetry may result instead (or also) from cells in different parts of the segment having differing abilities to degrade Wingless protein (J.-P. Vincent, Nat. Inst. Med. Res., London).

Protein degradation also affects the Hedgehog signal-transduction pathway. The proteins Patched and Smoothened were thought to be components of a cell-surface receptor complex that is activated by Hedgehog protein to send signals inside the receiving cell. But it seems that this view may be simplistic. Although levels of *smoothened* messenger RNA are uniform across the epidermis and wing imaginal disc (the structure from which the wing will form), levels of Smoothened protein vary (S. Cohen, EMBL, Heidelberg; M. Noll, Univ. Zurich) (Fig. 1a, overleaf). This variation occurs because Patched promotes the degradation of Smoothened protein, except in cells that receive the Hedgehog signal. In these cells, Hedgehog-dependent removal of Patched from the cell surface results in increased phosphorylation of Smoothened and its accumulation at the cell membrane. These interactions complicate conventional models of receptor-mediated signalling.

So the protein-degradation machinery is required for normal regulatory processes, but it sometimes goes awry, with pathological consequences. Versions of the human protein Ataxin-A that have extra-long tracts of glutamine residues cause spinocerebellar ataxia type 1 (ref. 2). The expression of such human proteins in flies produces several defects seen in the human disease, including neuronal degeneration (J. Botas, Baylor Coll. Med., Houston). Mutations that disrupt components of the cellular 'ubiquitin-mediated' protein-degradation machinery suppress these defects. So, disease-causing Ataxin-A proteins may wreak their havoc by overloading the normal neuronal protein-degradation apparatus.

Protein modification, as well as turnover, is important in coordinating many signalling pathways, including the Notch pathway, discussed in these pages recently[3]. In places where the Notch protein establishes borders between groups of cells, its activity is regulated by Fringe (Fig. 1b, overleaf), an enzyme that adds particular sugar groups to Notch (and possibly to Notch's ligands; K. Irvine, Waksmann Inst., Piscataway). A hint of further protein modification in Notch signalling comes from the localization of the Mastermind protein, a long-mysterious component of the pathway, to

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so-called PML bodies in the nucleus (S. Artavanis-Tsakonas, Mass. Gen. Hosp., Boston). These bodies are associated with 'SUMO modification'[4] — a 'tagging' process that singles proteins out for degradation.

Signal transduction often involves protein modification by phosphorylation. Visualization of this process is helping to unravel the complexity of signalling from bone morphogenetic proteins (BMPs). A gradient of BMP proteins is thought to produce different cell fates both along the dorsal–ventral embryonic axis and across the wing[5]. The phosphorylation of Mad or Smad proteins is a key step in transmitting the BMP signal to the nucleus. Monitoring of Mad/Smad activation[6] does reveal a gradient in the developing wing but, unexpectedly, there is no indication of graded activation of Mad/Smad along the embryonic axis (B. Shilo, Weizmann Inst.; T. Tabata, Univ. Tokyo; L. Raftery, Mass. Gen. Hosp., Boston). Instead, activation is detected only in a dorsal stripe of cells, whose fates require the highest levels of BMPs. This leaves unanswered the question of how signals from BMPs are propagated through the cells in which activation of Mad/Smad cannot be detected.

Signal-transduction pathways may also be influenced by the regulated targeting of their components to the nucleus. Nuclear localization of phosphorylated mitogen-activated protein kinase is the final step in an intracellular signalling pathway that begins with the activation of a 'receptor tyrosine kinase' enzyme. The Corkscrew protein appears to control this final step by affecting the recruitment of importin (L. Perkins, Mass. Gen. Hosp., Boston), which carries proteins into the nucleus. A similar intersection with the nucleus-to-cytoplasm transport machinery affects the transcription factors Dorsal and Dif (C. Samakovlis, Umeå Univ., Sweden), which are involved in *Drosophila* immunity.

It is still too soon for the full impact of the recently completed *Drosophila* genome sequence to be felt. But one post-genomic revelation is the prevalence of gene families in *Drosophila*. For example, Warniu — a new member of the Snail protein family — is, like its two siblings, present in neural progenitor cells (T. Ip, Univ. Massachusetts). There are seven relatives of Wunen (R. Lehmann, Skirball Inst., New York), an enzyme that influences germ-cell migration[7]. A protein involved in ensuring that growing neuronal axons do not recross the embryonic midline is the receptor Roundabout[8]. Two more Roundabout-like proteins have now come to light, one of which also participates in the decision of axons to cross the midline (B. Dickson, Inst. Mol. Pathol., Vienna). Intriguingly, it appears that, after crossing the midline, the axons select their lateral position according to the combination of Roundabout proteins that they express.

The importance of regulated gene expression in development is undeniable. But our understanding of fly development must also incorporate changes in the stability, activity and localization of key proteins (and mRNAs too, as illustrated by their dramatic localization within the embryo; I. Davies, Univ. Edinburgh; D. Ish-Horowicz, Imperial Cancer Research Fund, London; H. Krause, Univ. Toronto). We now need to develop more techniques to look at both spatial (subcellular) and temporal mechanisms for coordinating protein activities and cell behaviours. The result will be an increasingly four-dimensional view of *Drosophila* development.

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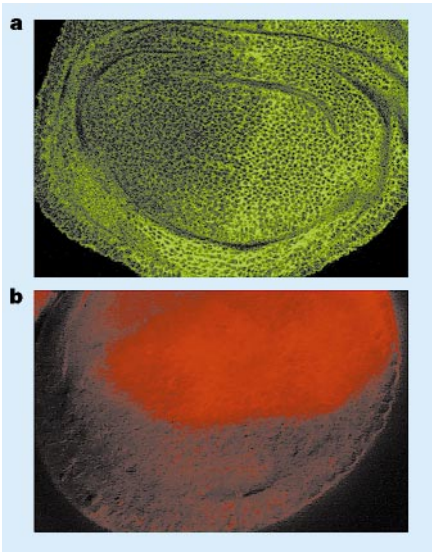

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Figure 1 **The importance of protein modification in *Drosophila* development.** a, **Levels of the Smoothened protein (green staining) vary across the wing imaginal disc. These levels are regulated by protein turnover[9].** b, ***fringe*** **messenger RNA (red staining) is expressed more highly in the dorsal part of the wing disc. The Fringe protein adds particular sugar groups to the Notch receptor, modifying its interactions with its ligands. So this asymmetric distribution of *fringe* mRNA results in modification of Notch only in certain parts of the wing disc.**

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Daedalus

The sound of silence

The mobile phone, that essential modern accessory, makes its user immediately unpopular with those around him. For some reason, talking into such a phone is far more annoying to external listeners than a conversation with a human companion, or even soliloquial muttering. Daedalus reckons that the phone user instinctively projects his voice to reach the distant party. He is now inventing a phone which can be spoken into silently.

Speech is formed by the mouth and tongue acting as an ever-changing resonant cavity for tones produced by the larynx. The tones themselves are very basic; someone who has lost his larynx can speak intelligibly with a simple buzzer as a replacement. Daedalus's brilliant idea is to provide an ultrasonic 'buzzer' as a larynx. His 'Ultraphone' has a narrow pipe, like a drinking straw, which projects into the user's mouth and injects a set of inaudible ultrasonic frequencies into it. The user whispers or mouths his speech silently, and a microphone detects the modulations imposed by his mouth and palate on the ultrasonic signal. A heterodyne circuit downshifts this signal into the audio range, thus reconstituting the speaker's normal voice, and transmits it to the called party. Like a normal telephone, it also injects a proportion of the speaker's reconstituted speech back into his own earpiece as a 'side-tone' for aural feedback. Thus he hears his voice quite normally, and is not tempted to speak audibly. Indeed, any attempt to do so will result in strange distortions as the audio is downshifted and aliased by the heterodyne circuit.

This simple system would produce a flat and toneless speech, rather like that of the laryngectomy patient with his buzzer. But Daedalus hopes to equip the Ultraphone with a program that recognizes the tonal clues implicit in silently mouthed speech, and varies the ultrasonic frequencies in sympathy. This should give far more realistic speaking tones, close to the user's natural voice.

The Ultraphone will sweep the market. Yuppies and poseurs will be able to make truly silent phone calls anywhere, even in concert performances and prayer meetings, without disturbing the proceedings or revealing the important, confidential matters they are discussing. And even in a boiler factory or gunnery range, ambient noise will not distract them. High above the audible clamour, their ultrasonic deliberations will travel clear and unaccompanied. **David Jones**